Validation of the of Mid-Thigh Bone, Muscle and Fat Mass by Densitometry as a Sensitive and Practical Region of Interest for the Diagnosis of Tissue Loss Syndromes

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Research Article

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Abstract

Purpose: The prevalence of musculoskeletal tissue loss syndromes (osteoporosis and sarcopenia), as well as obesity and their combinations, is on the rise. Previous reports indicate mid-thigh dual-energy x-ray absorptiometry (DXA) is well-suited for the simultaneous assessment of bone, muscle, and fat mass in a single scan.

Methods: Using DXA images of 1322 community-dwelling adults (57% women, age 58.2 ± 15.6 SD), bone, lean and fat mass were quantified in five regions of interest (ROIs): a) a 2.6 cm; and b) a 13 cm thick slice of mid-thigh; c) whole thigh; d) whole calf; and e) forearm. Conventional indices of tissue mass i.e. appendicular lean mass, hip, and spine bone mineral density (BMD) and total, gynoid and android fat mass, were also calculated. Their associations with muscle strength (handgrip strength) and performance (timed-up-and-go [TUG] and gait speed), as well as falls and fractures, were investigated.

Results: Lean mass in all ROIs was well-correlated with outcomes, and according to regression analyses, lean mass explained two-thirds, a third and a quarter of the variability in the handgrip strength ($r^2 = 0.63$ to 0.64 , $p < 0.001$), TUG ($r^2 = 0.34$ to 0.37 , $p < 0.001$) and gait speed ($r^2 = 0.25$ to 0.27 , $p < 0.001$), respectively. Lean masses were negatively associated with falls only when corrected for BMI. In the ≥ 60 yo cohort, only mid-thigh lean mass corrected for BMI was associated with decreased odds of falls (24%, $p = 0.029$). In all assessed ROIs, BMD was associated with the number of incident fractures within five years (OR = 0.72 to 0.93 , $p = 0.034$ to < 0.001). Increased fat mass was associated with increased TUG time and decreased odds of fractures. Muscle indices corrected for BMI performed better, compared to those uncorrected or corrected for height².

Conclusion: Compared to the conventional indices and four other ROIs, mid-thigh tissue masses were associated with markers of muscle performance. Mid-thigh BMD and lean mass were superior and showed more consistent and more robust associations with adverse outcomes (falls and fractures), particularly in the older population.

Mini Abstract

Using DXA images of 1322 community-dwelling adults, bone, lean and fat mass were quantified in five regions of interest (ROIs). Their associations with muscle strength, muscle function, falls and fractures were investigated. Compared to the conventional indices and four other ROIs, mid-thigh tissue mass measurements were associated with markers of muscle performance. Mid-thigh BMD and lean mass were superior and showed more consistent and more robust associations with adverse outcomes (falls and fractures), particularly in the older population.

Introduction

The aging population is expanding worldwide, which also associates with a higher occurrence of chronic diseases. Hence, the prevalence of age- or disease-associated tissue loss syndromes (e.g. sarcopenia,

osteoporosis, osteosarcopenia, cachexia, eating disorders, and frailty) and fat mass abnormalities (e.g. obesity, sarcopenic obesity, diabetes) and their associated adverse outcomes including falls and fractures are on the rise [1,2]. Bone, skeletal muscle, and fat are closely interconnected not only anatomically but also embryonically (mesodermal in origin), mechanically, metabolically, and chemically [3-5]. Furthermore, pathophysiological, and epidemiological research indicates that the decline of bone or muscle can impact the volume of the other, and both tissues are replaced with ectopic fat that plays an active role in the deterioration of both tissues [6,7].

The combined effect of osteoporosis and sarcopenia – termed “the hazardous duet”, increases older people’s susceptibility to falls and fractures, which are among the leading causes of morbidity and mortality in this population [8-11]; thus, representing a significant personal and public health burden [12, 13]. Osteosarcopenic individuals are at a significantly higher risk of morbidity and mortality than those with either of the diseases alone [14,15]. In addition, nearly two-thirds of older inpatients and up to 80% of nursing home residents [16, 17] and up to 79% of community-dwelling older adults [18] have at least one of the tissue loss syndromes exemplified above. Overall, the impact of these conditions on health includes reduced independence and life participation and increased disability [19].

The concomitant decline of bone and muscle, with or without changes in fat mass and distribution, is not limited to older adults. Within just 48 hours, patients admitted to an intensive care unit (ICU) could lose 1 kg of their muscle mass and 2% of their bone mass [20], and tissue loss syndromes are still a significant concern in younger eating disorder patients or those with malignancies and other acute or chronic conditions. Irrespective of the cause, tissue loss syndromes are expensive and painful, debilitating, and a major cause of disability, inactivity, and depression [19-22]. Furthermore, in medical inpatients of all ages, these conditions predict prolonged stay, poor functional recovery after hospitalization, and resistance to interventions (i.e. nutrition and exercise) [21-23]. Hence, timely, affordable, and quick assessment of muscle, bone, and fat in a wide range of conditions is mandated.

Assessment for sarcopenia generally includes muscle mass, strength and physical performance in different combinations depending on which definition of sarcopenia is chosen [24]. The current gold-standard for the diagnosis of osteoporosis/osteopenia is based on dual-energy x-ray absorptiometry (DXA) to assess bone mineral density (BMD) at the femoral neck, lumbar spine, and forearm, which requires several scans, is time- and resource-consuming, and is not always optimal to assess for the presence of other concurrent tissue loss syndromes. The mid-thigh region of interest (ROI) has recently been recognized as a clinically relevant and cost-effective potential site to screen for bone and muscle health by the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) [25]. Also, there is evidence that tissue masses (bone, lean and fat mass) in the mid-thigh are better associated with performance and the adverse outcomes of sarcopenia and osteoporosis in older persons at high risk of falls and fractures [26]. Therefore, we hypothesized that in the general population, and very importantly, in community-dwelling older adults, mid-thigh tissue masses are as good or better predictors of clinical outcomes compared to tissue masses in the conventional indices of tissue mass and tissue masses in three other ROIs (whole thigh, whole calf, and whole forearm).

Materials And Methods

Participants – Based in Geelong, Victoria (Australia), the Geelong Osteoporosis Study (GOS) is an ongoing population-based prospective cohort study that continues to investigate the epidemiology of osteoporosis since 1993 [27]. Participants were randomly selected using electoral rolls for the Barwon Statistical Division (BSD) surrounding Geelong. For most complete data availability reasons, this study utilized DXA images and data from the 15-year follow-up phase of the GOS (conducted 2010-2014 for the female cohort and 2016-2019 for the male cohort)). Images and data for 1322 community-dwelling adults were available, including 748 out of 894 female participants aged 28-91. Similarly, imaging data were available from 574 out of the 624 male participants aged 31-96 years. The demographics of the sub-study population is presented in Table 1.

Measurements – GOS collected clinical, biochemical, questionnaire and imaging data from participants throughout the study and the follow-up periods. Specific details can be found in the Cohort Profile [27]. The variables that are relevant to our research include anthropometric measures (weight and height), physical performance measures (timed up-and-go [TUG], handgrip strength, and gait speed), whole-body, femur and spine DXA images and falls and fractures history questionnaires. One-year retrospective falls history and 5-year retrospective fracture history confirmed via radiology clinics of the catchment area were used.

Image Acquisition and Analysis – Whole-body, spine and hip DXA scans of all participants were acquired using a GE Lunar Prodigy encore™ machine (GE Medical Systems Lunar, Madison, WI, USA), and BMD and body composition (appendicular lean mass [ALM] and fat mass in standard ROIs [total body fat, fat percentage, android and gynoid fat]) were determined using custom analysis software (version 14.10.002), as per manufacturer's standard protocols. Additionally, ten new ROIs (five symmetrical pairs) were defined and analyzed on whole-body scans: ROIs 1&2, 2.6 cm thick and ROIs 3&4, 13 cm thick at left and right mid-thigh – defined as the mid-point between the upper margin of the greater trochanter of the femur and the lower margin of the medial femoral condyle (Figure 1). ROIs 5&6 for the left and whole right thigh, defined as the region between the lower margin of the ischial tuberosity and the femoral condyles' lower margin. ROIs 7&8 for the left and whole right calf, defined as the region between the upper margin of the tibial plateau and the upper margin of the tibiotalar joint. ROIs 9&10 for the left and right forearm are defined as the region between the lower margin of the olecranon and the lower margin of the radiocarpal joint (Figure 1).

Bone, lean, and fat mass were estimated for each region using the DXA custom software analysis machine. All ROIs with metallic artifacts or participant positioning issues, causing an overlap of ROIs with android/gynoid fat or other regions, were excluded from analyses. The average values of right and left ROIs were used for statistical analysis – resulting in 5 ROIs to be compared with conventional indices: 2.6 cm mid-thigh ROI, 13cm mid-thigh ROI, whole thigh ROI, whole calf ROI and whole forearm ROI. The correlations between the indices calculated for the new five ROIs vs the conventional indices were sought. Also, bone, lean and fat mass in all ROIs (where necessary, corrected, and uncorrected for height² and

body mass index (BMI)) were used to predict outcomes of interest listed below. All measures were collected by trained personnel:

Handgrip strength – Handgrip strength was measured using a hand-held analog dynamometer (Jamar, Sammons Preston, Bolingbrook, IL, USA) for women and a digital dynamometer (Vernier, LoggerPro3) for men. The testing procedure was demonstrated to participants before the measurement trials. With the participant seated in a comfortable position and the arm holding the dynamometer flexed at the elbow to 90 degrees, the participant was asked to squeeze the device as hard as possible for several seconds, and the peak reading was recorded. This procedure was repeated for each hand. For women, the readings were performed in duplicate on each hand with no time interval between trials, and for men, trials were repeated in triplicate on each hand, holding the peak for 3 s with a 5-s interval between trials. The mean of the maximum value for each hand was used in further analyses. Measures from the Vernier device were transformed to Jamar equivalent values according to the following equation: $HGS_{Jamar} (kg) = 9.50 + 0.818 * HGS_{Vernier} (kg) + 8.80 * Sex$, where sex = 1 for men, which was developed by measuring the maximum HGS on each device for 45 adults aged 21–67 years.

Gait assessment – For men only, usual gait speed (m/s) was determined by measuring the time taken (in seconds) to walk a distance of 4 m.

Timed Up and Go test (TUG) – The TUG test was performed using a 40 cm high chair, with participants asked to stand from the chair and walk 3 m at their normal pace to a marked line before returning to the starting position and sitting.

Falls and fractures – Falls were defined as “when you suddenly find yourself on the ground, without intending to get there, after you were in either a lying, sitting or standing position” [28, 29]. Self-reported falls for a year pre-DXA scan were recorded using a questionnaire. Falls were documented by asking the participant 1) whether they have suffered a fall and 2) the number of experienced falls during the year before the day of the assessment. The occurrence of fractures within the last five years (prior to DXA scan) was assessed in a retrospective manner, confirmed from radiology clinics in the study region. Fragility fracture was defined as a fracture that result from mechanical forces that would not ordinarily result in fracture.

This study was approved by the Barwon Health Human Research Ethics Committee (HREC Reference Number: 92/01_E7 and 00/56_E7). All participants provided informed, written consent. Collaboration between Melbourne University and Barwon Health was reached to share the data from GOS to be used for analysis that made this study possible (collaboration agreement number: TP709555).

Statistical Analysis – Descriptive statistics are presented as mean $\pm$ standard deviation. Correlations between the indices obtained from selected ROIs and conventional indices were estimated using Pearson's correlation coefficient. Linear regression was used for physical performance outcomes, while logistic regression was used for falls and fractures (coded as any vs none). To ease comparison across models, all predictors were transformed using natural logarithm, and results expressed as change in

outcome (with 95% confidence intervals) associated with a 10% increase in the predictor. Some outcome variables (e.g. TUG) were transformed using a natural logarithm to improve model fit; results are expressed as fold change with a 10% increase in the predictor. Models were contrasted by comparing r^2 and root mean squared error (for linear regression) and AUC (for logistic regression). Two-tailed p-values with values <0.05 are considered significant. All statistical analyses were performed using Stata 16.1. All analyses were repeated for the whole sample, as well as those 60 years old and above.

Results

The summary of the study population's demographics and the main variables have been presented in Table 1. The fit of the model of new ROIs was equivalent to conventional ROIs (similar root mean squared error). Only the associations adjusted for age and sex are presented here.

Associations between muscle indices and clinical outcomes:

In the whole sample (including all subjects under and over 60), lean mass in all ROIs was associated with handgrip strength (10% increase in the lean mass was associated with 0.8-1.5 kg increase in handgrip strength, $p<0.001$). They explained around two-thirds of the handgrip strength variability ($r^2 = 0.62$ to 0.64 , $p<0.001$).

Although with or without correcting, lean mass was associated with handgrip strength, correcting lean mass for BMI was associated with almost twice as strong associations with handgrip strength in any region for the whole population (compared to correcting for height² - except for forearm). Similar trends were observed for those over 60 years; however, these associations were consistently significant only when corrected for BMI.

Over a third of the variability in TUG ($r^2 = 0.34$ to 0.37 , $p<0.001$) and just over a quarter of variability in gait speed ($r^2 = 0.25$ to 0.27 , $p<0.001$) could be explained by the variability in lean mass of all ROIs; and the strongest associations were observed using mid-thigh ROIs (10% increase in lean mass was associated with 3.5% decrease in TUG and with 2.5% increase in gait speed). In the 60+ subset of the population, lean mass in all regions explained slightly less of the variance in TUG and gait speed ($r^2 = 0.28$ - 0.34 and 0.21 - 0.24 , respectively). In this subset, mid-thigh lean mass produced the most consistent results where for every 10% increase in lean mass, TUG time decreased by $>4.3\%$ and gait speed increased by $>2.4\%$. All associations were significant only when corrected for BMI (not h^2).

In the whole sample, ALM, both mid-thigh lean mass indicators (2.6 and 13 cm slices) and whole thigh lean mass were associated with falls only when corrected for BMI. With 10% increase in mid-thigh lean mass, odds of falls decreased by 13% ($p = 0.002$); and by 9-10% per 10% increase in ALM or whole thigh lean mass ($p = 0.016$ and 0.027 , respectively). However, given an AUC of only 0.65, all lean mass indices are not strong indicators of past falls.

In the 60+ cohort only with higher mid-thigh lean mass (both 2.6 and 13 cm slices), and only when corrected for BMI, odds of falls decreased by almost a quarter (23% and 24%, $p= 0.029$ and 0.054), respectively.

None of the lean mass indicators was associated with fractures in the whole sample or those 60+ years old. Nevertheless, only with increasing mid-thigh lean mass, odds of hip fractures increased in the general population (and not the 60+ subset; $OR= 1.7$, $p< 0.003$, $AUC= 0.78$).

Bone Indices:

BMD in all ROIs except the lumbar spine was associated with grip strength (10% increase in BMD was associated with 0.18-0.75 kg increase in grip strength, ($r^2= 0.6$, $p< 0.037$). BMD only in the mid-thigh and whole-thigh ROIs was associated with TUG (increase in 10% of bone mass associated with a decrease of 1.9-2.5% in TUG ($r^2= 0.3 - 0.4$, $p\leq 0.002$), with the strongest association in 13 cm mid-thigh slice.

Only mid-thigh BMD (both 2.6 and 13 cm slices) was associated with increased handgrip strength and decreased TUG in the older cohort. Every 10% increase in mid-thigh BMD was associated with 0.44 and 0.49 kg higher handgrip strength and 2.0 and 2.6% faster TUG time. None of the BMD indices showed a significant association with gait speed or falls in either total or subset populations.

In both whole population and older subset, BMD in all ROIs was associated with any fractures within 5 years ($OR= 0.68$ to 0.93 , $p= 0.034$ to <0.001 , $AUC= 0.58$ to 0.65) - except whole forearm and 2.6 cm slice of the midthigh. BMD in all ROIs (except whole forearm) was also associated with decreased risk of fragility fracture ($OR= 0.64$ to 0.84 , $p= 0.018$ to <0.001 , $AUC= 0.60$ to 0.67). Similarly, in both populations, all BMDs were associated with decreased risk of hip fracture ($OR= 0.47$ to 0.84 , $p= 0.028$ to <0.001 , $AUC= 0.81$ to 0.86 ; except in mid-thigh, and marginally in spine), and vertebral fracture ($OR= 0.64$ to 0.83 , $p= 0.039$ to <0.001 , $AUC= 0.68$ to 0.74 ; except in femoral neck, hip whole calf, and forearm).

Fat indices:

Fat indices did not show associations with handgrip strength except when thigh and calf regions were corrected for height²; a 10% increase in fat mass was associated with around a 0.1 kg decrease in handgrip strength ($r^2= 0.6$, $p< 0.035$). Similar trends were observed for the associations with gait speed, where a 10% increase in fat mass (whole thigh, calf, or forearm) was associated with a 0.5 cm/s increase in gait speed ($r^2= 0.25$, $p\leq 0.05$). In the 60+ subset, none of the fat indices was associated with handgrip strength and only forearm fat was weakly associated with decreased gait speed (0.5 cm/s decline per 10% increase in forearm fat mass, $r^2= 0.22$, $p= 0.024$).

All fat mass indices (uncorrected, corrected for height² or BMI) were associated with TUG. A 10% increase in fat mass was associated with an increase in TUG of 0.5% ($r^2= 0.3$, $p< 0.02$). Similarly, in the 60+ group, irrespective of the region, and uncorrected or corrected for height², fat mass was associated with increased TUG time (0.78 to 1.11% for every 10% increase in fat mass, $r^2= 0.29$ to 0.35 , $p\leq 0.001$).

None of the fat indices was associated with past falls, and only forearm fat mass showed some association with past fractures (a 10% increase in fat mass was associated with a 4% decrease in odds for fractures (AUC= 0.62, $p= 0.012$)). No such associations were observed in those 60+ years-old.

Discussion

Our data suggest that bone indices in all five ROIs are moderately associated with the conventional indices, while lean and fat mass indices are highly correlated with their conventional counterparts. Compared to the conventional and other new indices of tissue mass (as internal controls), mid-thigh ROI lean, bone, and fat mass (particularly the larger 13 cm slice) are as well, or more strongly associated with strength, performance, falls and fractures. Correcting lean mass for BMI (particularly in the mid-thigh) and fat mass for height² also associated better with the outcomes. An increase in the fat mass of most ROIs (as an indicator of obesity) is associated with strength and performance decline.

A recent pilot study reported that mid-thigh has significant potential in assessing bone, lean, and fat mass in a “one-stop” DXA scan, with a fraction of time and radiation dose, compared to the required multiple conventional scans without needing difficult positioning [26]. Our results also provide further evidence that mid-thigh tissue masses show non-inferior to superior associations with outcomes that are of clinical interest in obesity and tissue loss syndromes, particularly in older adults.

This study is of direct clinical relevance, as techniques that can better and quickly estimate tissue loss syndromes (e.g. osteosarcopenia, cachexia, malnutrition, eating disorder-associated weight loss or frailty) and fat mass abnormalities (e.g. obesity, sarcopenic obesity) are highly in demand. Current research results indicate that mid-thigh lean mass, when corrected for BMI, shows comparable or slightly better associations with outcomes than ALM (the established index), which is in line with previous reports of the better performance of the mid-thigh lean mass in predicting performance and adverse outcomes in older adults [26].

Furthermore, all tested lean mass indicators corrected for BMI (including ALM/BMI, recommended by Foundation for the National Institutes of Health; FNIH [30]) show better associations with muscle performance compared to those corrected for height² (including ALM/h² recommended by the European Working Group on Sarcopenia in Older People (EWGSOP2) [25]). Myocyte metabolism/viability and muscle quality/strength are significantly affected by the degree of fat infiltration, and the resultant local lipotoxicity and the general inflammatory state associated with obesity and insulin resistance [31-33]. Therefore, correcting tissue volumes for BMI produces more relevant associations with muscle strength than correcting only for height, which is consistent with our previous report [26]. Further support for this conclusion comes from the negative association of fat indices with outcomes of interest, particularly in the older (60+) cohort.

Reportedly, changes as little as 6.5 kg for handgrip strength [34], 0.04 to 0.06 m/s in gait speed [35] and a decline of 1.1 seconds in TUG (data available for only those with osteoarthritis)[36] are considered

minimally meaningful to clinically significant. Therefore, the changes in handgrip strength (0.8 to 1.5 kg), gait speed (0.025 m/s), and TUG (0.1-0.3 seconds) per 10% higher muscle mass corrected for BMI (particularly in the mid-thigh) reported in this study, although considerable, are not clinically significant. Hence, assuming only muscles as the determinant of performance, 20, 40 to 80 and over 100% increase in lean mass would be required for significant increases in gait speed, grip strength and TUG, respectively. This is contrary to our findings in a population of older people at high risk of falls and fractures, where even small changes in lean mass, especially in the mid-thigh, were associated with highly significant improvements in performance and strength [26]. This finding emphasizes the particular importance of lean mass maintenance in older people.

The mid-thigh ROI has recently been recognized as a potential site to screen for sarcopenia by EWGSOP2 that is clinically relevant and cost-effective [25]. Estimates of muscle mass determined from mid-thigh ROI by MRI scan are less variable and have a higher correlation with whole-body muscle mass than other ROIs [37]. DXA-derived mid-thigh lean mass is strongly correlated with cross-sectional muscle area (CSA) determined by computed tomography (CT) of the same region [38].

Our results also resonate with those of Zemel et al. [39]. They showed that measuring distal, mid-distal and mid-femoral BMD of those under 18 (acquired in lateral projection) is valid for clinical practice. Due to significant associations between BMD in this region with clinical outcomes, including response to bisphosphonate therapy and fractures in children. Indeed, the International Society for Clinical Densitometry adopted this method as an acceptable measure of BMD in children [40]. The convenience and usefulness of scanning the region in children, especially those with spinal abnormalities, cerebral palsy, or acute immobilization, is another benefit of the technique that resonates with geriatrics practice.

Interestingly, our data suggest higher bone mass (particularly in the mid-thigh) is associated with better grip strength, and fat mass indicators have opposite effects (particularly in older adults). This further emphasizes the intimate relationship between muscle, bone and fat tissues and health and the necessity to monitor them together - preferably in a single quick scan. As the mid-thigh contains the largest volume of muscle in the body and the femur is the chief weight-bearing bone, there is an anatomical basis for the associations described, and our observations are consistent with other studies [41-43]. There are no ligaments or relatively voluminous tendons in this region, and considering the similar x-ray attenuation constants between muscles, tendons and ligaments, the lean mass measurements are more likely to be representative of muscle at mid-thigh. In addition, biomechanically, the femur's center of bending is likely at mid-thigh or its proximity. Hence, according to Wolff's law, being the longest bone in the body, the femur tolerates high bending forces and, consequently, preserves as much bone strength and mass as possible. Therefore, it could be speculated that small bone density changes in this area may represent large changes in loading and general bone mass.

With technology adaptation, mid-thigh DXA scanning can take from a few seconds up to a minute (versus around 30 minutes for a complete set of DXA scans); and can be done efficiently in a sitting position. This is particularly ideal for those who find lying flat difficult or uncomfortable due to conditions such as

back pain, severe spinal abnormalities, vertebral fractures, heart failure, chronic lung disease, and paroxysmal positional vertigo, which are common in older patients. Additionally, being away from joints, the mid-thigh is not directly affected by periarticular changes such as osteoarthritic sclerosis (e.g. hip), osteophytes or spondylosis, or vertebral compression fractures. Also, due to the relatively round geometry of the mid-thigh muscles, bone and fat distribution, no particular positioning (such as pronation of the leg to make femoral neck perpendicular to the radiation axis) is required for this region (unlike mid-calf, forearm, or femoral neck/hip scans). In addition to three other new ROIs, it has previously been shown that mid-thigh scans are superior to the mid-calf scans of the same subjects [26]. Possibly due to the same reason and different positioning of the forearm in whole-body scans in the current study, forearm results swap from other ROIs, which could be due to the degree of superimposition of radius and ulna with muscle depending on the degree of pronation. Finally, radiation-wise, a set of hip, spine, forearm and whole-body scans - not considering repositioning or repeat scans - can expose a patient to an effective dose of 25-30 μSv [48]. Although we have not calculated the effective dose for a mid-thigh scan, we expect it to be far less (around 1-2 μSv) and exempts radiating viscera and gonads that are most sensitive to radiation and are exposed in whole-body, hip, and lumbar spine scans. Considering these advantages, standardization of bone, muscle and fat mass in the area and determination of T- and Z-scores for tissue masses can lead to a quick, low cost and low radiation screening tool for various tissue loss syndromes exemplified above.

This study benefitted from relatively large sample size and radiographically confirmed retrospective fractures history. We also investigated the usefulness of the ROI for older adults as well as the whole population with consistent results. However, this study was subject to a few limitations. To reduce the number of comparisons which may lead to an increased chance of finding random associations, we did not study men and women separately. Nevertheless, we adjusted the associations for age and sex. Prospective falls and fracture data were not available for the population at this stage. Future studies will benefit from such analyses. Our sample included community-dwelling adults, which may not represent older adults or those younger persons at the risk of tissue loss syndromes. However, this study resonates with the results of another smaller study on falls and fractures clinic subjects [26] and another conducted in children [44] that also reported that mid-thigh tissue masses were of considerable diagnostic value. As the resolution of standard whole-body scans (on which the new ROIs were studied) are lower than the classic hip and spine scans, custom scanners that can acquire high-resolution images of mid-thigh may show even better associations with outcomes examined in this investigation.

In conclusion, mid-thigh muscle, bone and fat mass are well associated with the standard measures and show similar or better associations with strength, mobility, and falls in a population of older persons at high risk of falls and fractures. Therefore, we recommend mid-thigh for screening and follow up studies and warrant further longitudinal research to explore age-associated changes in bone, muscle and fat mass and the predictive value of this ROI for adverse outcomes (i.e. falls and fractures). Adaptation of the technology to scan mid-thigh may create a low-cost, low-radiation and quick screening tool that can potentially replace the existing DXA scanning protocols for various tissues. In addition, our results provide

further evidence that correcting the lean mass of any ROI for BMI is superior to correcting for height² for the prediction of outcomes.

Declarations

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Conflict of interest: Ebrahim Bani Hassan, Fernan Munandar Putra, Sara Vogrin, Julie A Pasco,

Mark A Kotowicz, and Gustavo Duque, declare that they have no conflict of interest.

Availability of data and material: Data will be available upon reasonable request.

Code availability: Not applicable

Authors' contributions: All authors were involved in data acquisition and analysis. All authors participated in the drafting of the article, and all authors approved the final version to be published.

Ethical approval: This study was approved by the Barwon Health Human Research Ethics Committee (HREC Reference Number: 92/01_E7 and 00/56_E7).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent for publication: Not applicable

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Tables

Table 1. *Attributes of Study Population presented as mean ± SD.*

	Men (n = 574)	Women (n = 748)
Demographics		
Age (yr)	62.5 ± 14.6	54.9 ± 15.5
BMI (kg/m ²)	27.8 ± 4.2	28.2 ± 5.8
Physical performance		
Timed up and go (s), median (IQR)	8.4 (7.5, 9.8)	7.7 (6.6, 9)
Handgrip Strength (kg), mean (SD)	24.2 ± 7.8	38.8 ± 7.7
Gait speed (m/s), mean (SD)	0.95 ± 0.21	N/A
Adverse events		
Episodes of falls in the last year, N (%)	201 (26.9%)	83 (14.5%)
Episodes of fractures in the last 5 years, N (%)	67 (9.0%)	65 (11.3%)
Bone Indices - BMD (g/cm²)		
Neck of Femur (NOF)	0.970 ± 0.14	0.923 ± 0.18
Hip	1.05 ± 0.15	0.974 ± 0.15
Lumbar Spine	1.32 ± 0.20	1.206 ± 0.18
Mid-thigh ROI	1.99 ± 0.20	1.624 ± 0.20
Whole thigh ROI	1.61 ± 0.15	1.383 ± 0.15
Calf ROI	1.370 ± 0.17	1.149 ± 0.15
Forearm ROI	1.15 ± 0.17	0.910 ± 0.13
Muscle Indices		
ALM/h ² (kg/m ²)	8.49 ± 0.98	6.69 ± 0.84
ALM/BMI (m ²)	0.95 ± 0.15	0.65 ± 0.12
2.6 cm mid-thigh ROI lean mass (kg)	0.43 ± 0.07	0.32 ± 0.05
13 cm mid-thigh ROI lean mass (kg)	2.13 ± 0.34	1.56 ± 0.26
Whole thigh ROI lean mass (kg)	5.42 ± 0.87	3.74 ± 0.64
Calf ROI lean mass (kg)	2.32 ± 0.37	1.64 ± 0.28
Forearm ROI lean mass (kg)	1.16 ± 0.16	0.66 ± 0.09
Fat Indices (kg)		
Gynoid Fat Mass	3.84 ± 1.36	5.55 ± 1.90
Android Fat Mass	2.71 ± 1.15	2.71 ± 1.30
Total Fat Mass	6.55 ± 2.42	8.26 ± 3.07

2.6 cm mid-thigh ROI Fat Mass	0.15 ± 0.06	0.29 ± 0.11
13 cm mid-thigh ROI Fat Mass	0.77 ± 0.31	1.47 ± 0.57
Whole-thigh ROI Fat Mass	2.32 ± 0.84	3.70 ± 1.36
Calf-ROI Fat Mass	0.66 ± 0.27	1.11 ± 0.45
Forearm ROI Fat Mass	0.18 ± 0.09	0.23 ± 0.12

Tissue masses of all five new ROIs showed moderate to very strong associations with the conventional indices of bone (hip, neck of femur and spine BMD), muscle (ALM) and fat (android, gynoid and total fat; Table 2).

Table 2: *The correlations between all five new ROIs vs the traditional indicators of bone, muscle, and fat mass.*

<i>Bone Indices</i>					
	2.6cm mid-thigh	13cm mid-thigh	Whole thigh ROI	Whole calf ROI	Forearm ROI
	ROI (g/cm ²)	ROI (g/cm ²)	(g/cm ²)	(g/cm ²)	(g/cm ²)
SPINE	0.395	0.437	0.590	0.537	0.448
BMD					
(g/cm ²)					
NOF BMD	0.372	0.421	0.659	0.618	0.413
(g/cm ²)					
HIP BMD	0.431	0.494	0.745	0.672	0.485
(g/cm ²)					
<i>Muscle Indices corrected for BMI</i>					
	2.6m mid-thigh	13cm mid-thigh	Whole thigh ROI	Whole calf ROI	Forearm ROI
	ROI lean	ROI lean	lean mass/BMI	lean mass/BMI	lean mass/BMI
	mass/BMI	mass/BMI			
ALM/BMI	0.930	0.937	0.977	0.943	0.923
(m ²)					
ALM/h ²	0.572	0.571	0.554	0.488	0.536
(kg/m ²)					
<i>Muscle Indices corrected for height²</i>					
	2.6m mid-thigh	13cm mid-thigh	Whole thigh ROI	Whole calf ROI	Forearm ROI
	ROI lean mass/h ²	ROI lean mass/h ²	lean mass/h ²	lean mass/h ²	lean mass/h ²
ALM/BMI	0.287	0.571	0.541	0.488	0.647
(m ²)					
ALM/h ²	0.855	0.864	0.951	0.876	0.823
(kg/m ²)					
<i>Fat Indices uncorrected</i>					
	2.6cm ROI Fat	13cm ROI Fat	Whole-thigh Fat	Whole-calf Fat	Forearm Fat
	Mass	mass	Mass	Mass	Mass
Android	0.567	0.572	0.632	0.548	0.573
Fat (kg)					
Gynoid	0.931	0.936	0.958	0.862	0.654

Fat (kg)					
Total Fat	0.840	0.846	0.885	0.788	0.654
(kg)					
<i>Fat Indices corrected for BMI</i>					
	2.6cm ROI Fat	13cm ROI Fat	Whole-thigh Fat	Whole-calf Fat	Forearm Fat
	Mass/BMI	mass/BMI	Mass/BMI	Mass/BMI	Mass/BMI
Android	0.295	0.290	0.321	0.242	0.348
Fat (kg)					
Gynoid	0.792	0.791	0.801	0.672	0.494
Fat (kg)					
Total Fat	0.635	0.633	0.653	0.535	0.468
(kg)					
<i>Fat Indices corrected for height²</i>					
	2.6cm ROI Fat	13cm ROI Fat	Whole-thigh Fat	Whole-calf Fat	Forearm Fat
	Mass/h ²	mass/h ²	Mass/h ²	Mass/h ²	Mass /h ²
Android	0.509	0.511	0.573	0.498	0.523
Fat (kg)					
Gynoid	0.883	0.886	0.928	0.840	0.652
Fat (kg)					
Total Fat	0.785	0.788	0.841	0.752	0.645
(kg)					

Figures

Figure 1

Five pairs (ten) regions of interest (ROIs) were defined and analysed on whole-body scans. The average of right and left ROIs were used for analyses: ROIs 1&2 (purple), 2.6 cm thick and ROIs 3&4 (blue), 13 cm thick at left and right mid-thigh – defined as the mid-point between the upper margin of the greater trochanter of the femur and the lower margin of the medial femoral condyle. ROIs 5&6 (red) for the left and whole right thigh, defined as the region between the lower margin of the ischial tuberosity and the lower margin of the femoral condyles. ROI 7&8 (dark blue) for the left and whole right calf, defined as the region between the upper margin of the tibial plateau and the upper margin of the tibiotalar joint. ROI 9&10 (yellow) for the left and right forearm is defined as the region between the lower margin of the olecranon and the lower margin of the radiocarpal joint.